



# The role of RASSF proteins in modulating RAS driven lung tumors *in vivo*

Desmond Harrell Stewart<sup>1</sup>, Katherine Hobbing<sup>1</sup>, M. Lee Schmidt<sup>1</sup>, Howard Donninger<sup>2</sup>, Geoffrey J. Clark<sup>1</sup>

<sup>1</sup>Department of Pharmacology & Toxicology, <sup>2</sup>Department of Medicine, University of Louisville, Louisville, KY, USA

Correspondence to: Geoffrey J. Clark. Department of Pharmacology & Toxicology, University of Louisville, Louisville, KY, USA. Email: gjclar01@louisville.edu.

Provenance: This is an invited article commissioned by the Section Editor Chunlin Ou (Cancer Research Institute of Central South University, Changsha, China).

Response to: Blandino G, Lo Sardo F. In vivo experimental models account for higher complexity than in vitro preclinical settings in cancer. *J Thorac Dis* 2019;11:S461-4.

Submitted Mar 09, 2019. Accepted for publication Mar 14, 2019.

doi: 10.21037/jtd.2019.03.60

View this article at: <http://dx.doi.org/10.21037/jtd.2019.03.60>

Sir, the recent editorial of our article describing the first animal model for KRAS-driven lung tumors in a RASSF1A-deficient background (1) succinctly and accurately describes our work. I would like to take the opportunity to elaborate and to describe some additional pertinent experiments.

*Kras* may be the most frequently activated oncogene found in human cancer, but in addition to promoting transformation, aberrant activation of RAS can paradoxically lead to the induction of cell death by a variety of mechanisms, including apoptosis (2). In contrast, RASSF1A may be the most frequently inactivated tumor suppressor in human cancer (3). Strikingly, the most malignant and therapy-resistant human lung tumors almost always exhibit both activation of KRAS and suppression of RASSF1A (4).

When we initially cloned *RASSF1* in 2000 (5), we speculated that the protein might serve as a RAS-regulated tumor suppressor linking RAS to apoptotic signaling pathways. Thus, we predicted that loss of RASSF1A would uncouple RAS from growth inhibitory pathways, releasing the full transforming potential of RAS. When we finally performed the definitive experiment and generated a transgenic mouse system +/- for activated KRAS (*KRAS12D*) and RASSF1A, the results were gratifying as we observed a marked increase in lung tumor formation in the *RASSF1A*<sup>+/-</sup> animals (1). However, as mentioned in the editorial, we not only detected loss of apoptotic pathway signaling, but also observed up-regulation of all three classical RAS mitogenic pathways in tissue whenever RASSF1A was suppressed, even in the absence of oncogenic RAS. Surprisingly, the levels of pathway activation were

comparable between the *RASSF1A*<sup>+/-</sup> non-tumor tissue and the RAS-induced tumor tissue. This raises two questions: (I) how does RASSF1A suppress RAS-mediated mitogenic signaling, and (II) why is the loss of one allele of *Rassf1* alone—despite promoting mitogenic signaling—insufficient for tumor development?

RASSF1A has been implicated in modulating the AKT and MAPK pathways previously (6). We have also found that RASSF1A can complex with RALGDS, giving it a link to all three known RAS mitogenic signaling pathways. We now suspect an additional component may be at play. We have identified RASSF1A in direct, endogenous complex with a RAS GTPase Activating protein (RASGAP), and thus loss of RASSF1A may inhibit the GAP activity in a cell, resulting in the activation of non-mutant RAS proteins. This may be the first example of a feedback loop between a RAS effector and RAS-GTP levels.

As to why no tumors develop, perhaps the most intriguing possibility is that there may be an as yet overlooked RAS signaling pathway that is critical for *in vivo* transformation—yet not so for *in vitro* transformation—that is not activated by suppression of RASSF1A. This could be an inflammatory component, as we observed evidence of enhanced inflammation in the *KRAS*<sup>G12D</sup>/*Rassf*<sup>+/-</sup> tumors.

The experiment may also point to the true role of RASSF1A in tumorigenesis. When we commenced the transgenic experiment, we anticipated that we would obtain more tumors and that they would be more aggressive and more metastatic. We stopped the experiment when the first animals began to exhibit signs of distress. At this point,

pathology revealed no detectable metastasis to the liver. Moreover, although the average number of tumors was enhanced in the *KRAS<sup>G12D</sup>/RASSF<sup>+/-</sup>* mice, the average size was the same, irrespective of the RASSF1A status. The simplest explanation for this is that the most potent effect of RASSF1A suppression may be at the tumor initiation stage. Perhaps a cell undergoes a crisis if RAS signaling reaches a certain threshold and activating RASSF1A engages an apoptotic program to “edit out” the aberrant cell. In the absence of RASSF1A, this editing function is impaired and the cell survives, leading to more successful tumor initiation.

RASSF1A is a member of a family of 6 main members. Clearly these related proteins provide a degree of redundancy in RAS/RASSF signaling and share at least some biological properties. However, their roles in RAS-mediated transformation *in vivo* remain largely unknown. NORE1A/RASSF5 is the most closely related family member to RASSF1A and is also frequently suppressed by promoter methylation in lung cancer. *In vitro* studies showed us that NORE1A was a potent RAS senescence effector in HRAS-driven lung cancer cell systems *in vitro*. Moreover, suppression of NORE1A expression enhanced HRAS-driven colony formation of lung epithelial cells in soft agar (7). Mouse embryonic fibroblasts (MEFs) from transgenic mice knocked out for NORE1A could be fully transformed by KRAS alone, a process which usually also requires inactivation of p53 or Rb (8). However, when we repeated the transgenic KRAS lung tumor experiment with *NORE1A* knockout mice (generous gift from SB Lee, NIH), to our surprise, we detected no positive effect of NORE1A suppression on tumor formation. Indeed, we actually appeared to detect a small reduction in tumor formation. The reasons for this remain unclear to us. We have some evidence that KRAS preferentially couples to RASSF1A whereas HRAS preferentially couples to NORE1A. Also, while both RASSF1A and NORE1A can bind components of the apoptotic Hippo pathway (9), we have observed that RASSF1A preferentially effects YAP phosphorylation while NORE1A more strongly affects TAZ. Moreover, the shorter isoform of NORE1A has been implicated in immune function, and so immune effects could impact tumor development in NORE1A knockout animals. In any case, *in vivo*, RASSF1A and NORE1A suppression in an oncogenic RAS context exhibit very different effects.

Thus, we strongly agree with the premise of the editorial, that *in vivo* experiments will be required to accurately understand the biological role of the RASSF proteins in tumorigenesis, particularly in RAS-driven cancers.

## Acknowledgements

*Funding:* This work was supported by CA133171-01A2 (GJ Clark) and ES011564 (A1) (D Harrell Stewart).

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

## References

- Schmidt ML, Hobbing KR, Donninger H, et al. RASSF1A Deficiency Enhances RAS-Driven Lung Tumorigenesis. *Cancer Res* 2018;78:2614-23.
- Cox AD, Der CJ. The dark side of Ras: regulation of apoptosis. *Oncogene* 2003;22:8999-9006.
- Harada K, Toyooka S, Maitra A, et al. Aberrant promoter methylation and silencing of the RASSF1A gene in pediatric tumors and cell lines. *Oncogene* 2002;21:4345-9.
- Kim DH, Kim JS, Park JH, et al. Relationship of Ras association domain family 1 methylation and K-ras mutation in primary non-small cell lung cancer. *Cancer Res* 2003;63:6206-11.
- Vos MD, Ellis CA, Bell A, et al. Ras uses the novel tumor suppressor RASSF1 as an effector to mediate apoptosis. *J Biol Chem* 2000;275:35669-72.
- Thaler S, Hahnel PS, Schad A, et al. RASSF1A mediates p21Cip1/Waf1-dependent cell cycle arrest and senescence through modulation of the Raf-MEK-ERK pathway and inhibition of Akt. *Cancer Res* 2009;69:1748-57.
- Donninger H, Calvisi DF, Barnoud T, et al. NORE1A is a Ras senescence effector that controls the apoptotic/senescent balance of p53 via HIPK2. *J Cell Biol* 2015;208:777-89.
- Park J, Kang SI, Lee SY, et al. Tumor suppressor ras association domain family 5 (RASSF5/NORE1) mediates death receptor ligand-induced apoptosis. *J Biol Chem* 2010;285:35029-38.
- Donninger H, Schmidt ML, Mezzanotte J, et al. Ras signaling through RASSF proteins. *Semin Cell Dev Biol* 2016;58:86-95.

**Cite this article as:** Harrell Stewart D, Hobbing K, Schmidt ML, Donninger H, Clark GJ. The role of RASSF proteins in modulating RAS driven lung tumors *in vivo*. *J Thorac Dis* 2019;11(Suppl 9):S1436-S1437. doi: 10.21037/jtd.2019.03.60